IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Wong, C.-H.	
Serial No.:	09/077,712	Group Art Unit: 1654
Filed:	July 9, 1999	
For:	HIV Protease Inhibitors	**Declaration of Chi-Huey Wong under 37 CFR 1.131**
Examiner:	Meller, Michael V.	

DECLARATION

Hon. Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, Chi-Huey Wong, Ph.D. declare that:

1. I am the co-inventor, together with Deborah Slee and Karen Laslo, of the invention described and claimed in the above-referenced application and I am thus familiar with the subject matter described and claimed in that patent application.

2. I am aware that pending claims 19-26 within the above application has been rejected as anticipated under 35 U.S.C. 102(a) over a reference by Deborah Slee, Karen Laslo, John Elder, Ian Ollmann, Alla Gustchina, Jukka Kervinen, Alexander Zdanov, Alexander Wlodawer, and Chi-Huey Wong, Journal of the American Chemical Society, (December 1995), Vol. 117(33) pages 11867-11878.

3. I am a coauthor, along with Deborah Slee, Karen Laslo, John Elder, Ian Ollmann, Alla Gustchina, Jukka Kervinen, Alexander Zdanov, and Alexander Wlodawer of the above Moris-Varas reference.

4. I hereby declare that our invention was completed by Deborah Slee, Karen Laslo and myself prior to December 6, 1995, and that the invention occurred within the United States.

5. Attached hereto is a copy of the first page of a letter dated April 28, 1995 addressed to Dr. Jonathan Lasch from Donald G. Lewis referencing MS 9350-CH (Selectivity in the Inhibition of HIV and FIV Protease: Exploiting ne Pyrrolidine-Containing Core structure for the Development of Structure and Mechanism-based Inhibitors" (Wong, et al). Also attached hereto is a copy of the referenced MS 9350-CH. MS 9350-CH is a draft copy of a manuscript that was sent to the Journal of the American Chemical Society for publication and resulted in the publication of the reference by Slee, et al., viz., Journal of the American Chemical Society, (December 1995), Vol. 117(33) pages 11867-11878. The April 1995 date on the attached letter evidences the fact that the draft manuscript (MS 9350-CH) was completed prior to the publication of the Slee reference in December 1995 and evidences our completion of our invention prior to that date. The institutional address on the attached manuscript also evidences that the invention occurred within the United States.

6. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

9/5/03
Date

Chi-Huey Wong, Ph.D.

Disc 94-76

THE SCRIPPS RESEARCH INSTITUTE
10666 NORTH TORREY PINES ROAD
LA JOLLA, CALIFORNIA 92037
619 455-9100

April 28, 1995

Dr. Jonathan Lasch
Office of Technology Development
The Scripps Research Institute
La Jolla, CA 92037

Re: Disclosure 94-76: "α-Ketoamides as Inhibitors of Aspartyl Proteases" (Wong et al.)
MS 8650-CH: "α-Ketoamides Phe-Pro Isostere as a New Core Structure for the Inhibition of HIV Protease" (Wong et al.)
MS 9350-CH: "Selectivity in the Inhibition of HIV and FIV Protease: Exploiting new Pyrrolidine-Containing Core Structure for the Development of Structure and Mechanism-based Inhibitors" (Wong et al.)

Dear Dr. Lasch,

This letter is responsive to your request that OPC review the patentability of Disclosure 94-76. Disclosure 94-76 includes 3 parts, viz.:

Part 1: OTD's standard 3 page Technology Disclosure form;
Part 2: MS 8650-CH, attached to the Disclosure form; and
Part 3: 4 sheets of unnumbered supplemental diagrams, attached to the Disclosure form, detailing advanced embodiments of the invention.

In addition to MS 8650-CH, related manuscript, MS 9350-CH, is also discussed herein (Part 4).

Background:

MS 8650-CH was disclosed to PRI on 05/10/94, declined by PRI on 07/15/94, and published late 94. Disclosure 94-76 was submitted to PRI on 10/11/94. PRI's option for 94-76 expired on 01/11/95 without being picked up. PRI requested review of Disclosure 94-76 on 01/17/95, i.e., after its option had expired. MS 9350-CH is newly submitted herewith.

Donald G. Lewis, Esq., Ph.D.
Office of Patent Counsel, Associate Patent Counsel
Direct Line: 619 554-2937 FAX Line: 619 554-6312 Mail Drop TPC8

Selectivity in the Inhibition of HIV and FIV Protease: Exploiting new Pyrrolidine-containing Core Structures for the Development of Structure and Mechanism-based Inhibitors

Deborah H. Slee, Karen L. Laslo, John H. Elder, Chi-Huey Wong*

Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

Abstract

This paper describes the development of new pyrrolidine-containing keto-amide and hydroxyethylamine core structures as mechanism based inhibitors of the HIV and FIV proteases. It was found that the α-keto amide core structure 2 is approximately 300 fold better than the corresponding hydroxyethylamine[1] isosteric structure and 1300 fold better than the corresponding phosphinic acid[2] derivative as an inhibitor of the HIV protease. The keto-amide is however not hydrated in the absence of the HIV protease. Further analysis of the inhibition activities of modified pyrrolidine derivatives revealed that a *cis*-methoxy group at C- 4 would improve the binding 5 fold. Of the core structures prepared as inhibitors of the HIV protease, none of them shows significant inhibitory activity against the mechanistically identical FIV protease, and additional complementary groups are needed to improve inhibition.

Introduction

Human immunodeficiency virus (HIV) protease is an important target for inhibition. Though many potent *in vitro* inhibitors have been developed, most of them are either inactive or toxic *in vivo*, or the virus rapidly develops resistance.[3-5] The lack of animal systems to test the efficacy of the inhibitors further slows down the drug development process. Recently a similar protease has been identified in the life cycle of feline immunodeficiency virus (FIV)[6, 7] which is associated with a debilitating immunodeficiency syndrome in domestic cats, comparable to acquired immune

deficiency syndrome (AIDS) in humans. Studies have shown that up to 14% of the cats surveyed in the USA and Canada[8] and 28.9% in Japan[9] are infected with FIV. We are developing inhibitors to test against both HIV and FIV proteases, with the aim of developing selective inhibitors of these two mechanistically identical proteases. Another objective is to use cats as model systems on which to test HIV protease inhibitors *in vivo*.

HIV protease[10] is a 99 amino acid aspartyl protease[11] which functions as a homodimer. FIV protease is also a homodimeric aspartyl protease which consists of 116 amino acid residues.[12] Both HIV and FIV proteases are responsible for the processing of viral *gag* and *gag-pol* polyproteins into structural proteins and enzymes essential for the proper assembly and maturation of full infectious virions.[13] In particular HIV[14] and FIV proteases[15] show high specificity for the selective cleavage of the Tyrosine/Phenylalanine-Proline amide bonds in the Matrix-Capsid domain of the *gag-pol* polyproteins, a specificity not exhibited by mammalian cellular proteases which are not known to efficiently hydrolyze peptide bonds involving the proline nitrogen. It is this specificity that makes HIV-protease an attractive target for inhibition. Figure 1 compares the amino acid sequence about the matrix capsid cleavage site (Tyrosine ~ Proline bond) in both HIV and FIV. As can be seen the residues about the cleavage site are the same at four positions, P_3, P_1, $P_{1'}$ and $P_{2'}$. These similarities suggest that HIV-protease inhibitors may also inhibit FIV protease to some extent.

	P_4	P_3	P_2	P_1		$P_{1'}$	$P_{2'}$	$P_{3'}$	$P_{4'}$
HIV protease	**Ser**	**Gln**	**Asn**	**Tyr**	~	**Pro**	**Ile**	**Val**	**Gln**
FIV protease	**Pro**	**Gln**	**Ala**	**Tyr**	~	**Pro**	**Ile**	**Gln**	**Thr**

Figure 1. Amino acid sequence of the natural substrates for HIV and FIV proteases about the Y~P cleavage site.

Our group has recently developed a new keto-amide core structure[16] which appears to be more potent than any other mechanism-based isosteric core structures (such as those of the hydroxyethylamine[1] and phosphinic acid[2] derivatives) as an HIV protease inhibitor. Activated

ketones in general have been shown to inhibit aspartyl proteases such as renin,[17] and serine and cysteine proteases such as α-chymotrypsin and calpain respectively. Their modes of action however are not well understood.[18-21]

The activity of dipeptide isosteres is often enhanced by addition of amino acids residues to both the N- and C-terminus of the isostere to improve binding in the active site. Although this approach often provides very high binding affinity to HIV PR and some inhibitors of this type have been in clinical trials,[3] the resulting inhibitors generally exhibit metabolic instability and/or poor oral bioavailability.[22-24] It is possible to develop potent small molecule HIV-protease inhibitors that span P_1-P_2' subsites in HIV protease.[2526] In order to enhance the activity of the core isosteres containing either an α-keto amide moiety or a hydroxyethylamine, without the addition of amino acid side chains, we have investigated the effects of protecting groups and the substituents about the proline ring. This study lends itself well to a combinatorial approach as illustrated in Figure 2.

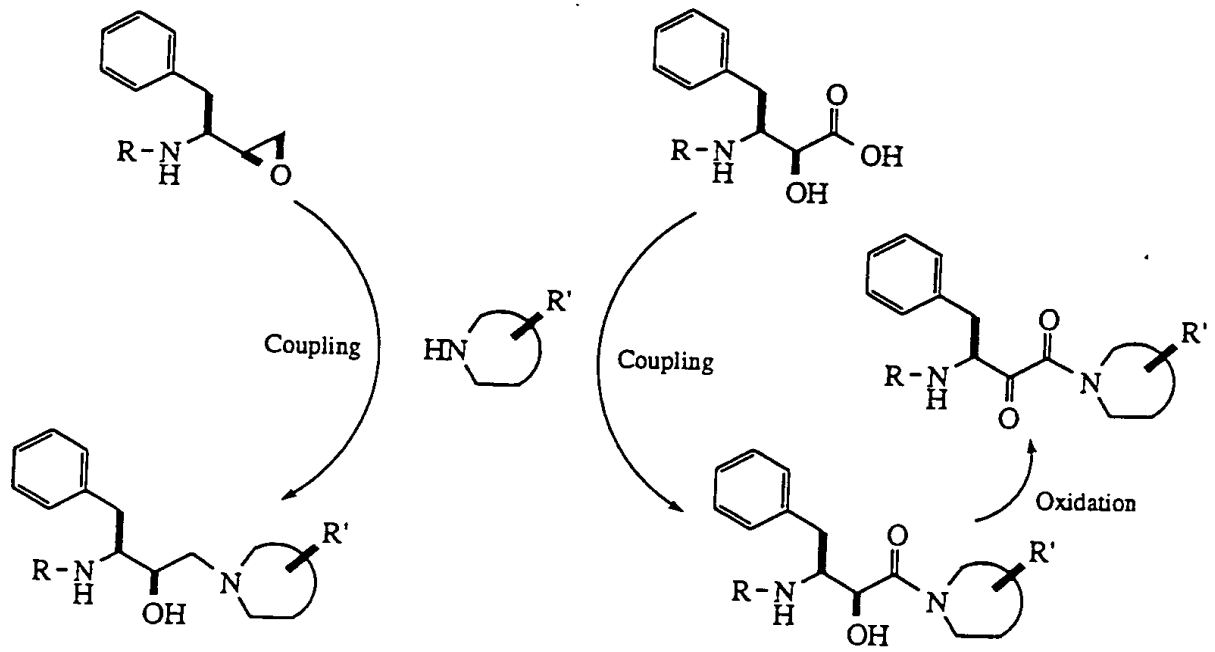
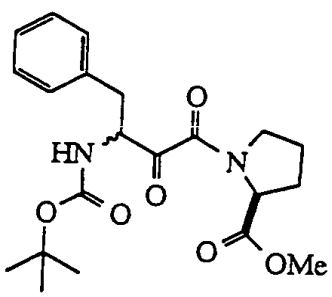


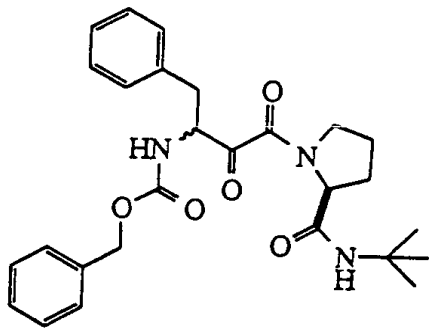
Figure 2. Illustration of how a combinatorial synthetic approach can be used to rapidly access a number of potential inhibitors of HIV and/or FIV proteases to determine the protecting group and ideal substitution pattern of the 'proline' moiety to provide maximum inhibition of the enzymes.

Results and Discussion

When assayed against HIV protease the novel α-keto amide **1** (Figure 3) was found to have a K_i of 6 μM. Subsequent studies have shown that a simple modification of the N- and C-terminal protecting groups to give **2** (Figure 3) enhances the potency of this core isostere against HIV protease, to give a K_i of 214 nM.



1 $K_i = 6\mu M$



2 $K_i = 214$ nM

Figure 3.

We attribute the increase in activity in compound **2** to favorable hydrophobic interactions between the phenyl ring of the Cbz-protecting group and the active site of HIV protease. The BOC-protecting group is also hydrophobic but is shorter and sterically more bulky making it unable to extend effectively into the appropriate hydrophobic binding pocket. All these factors contribute to making compound **1** a less potent inhibitor of HIV protease than **2**. This result demonstrates how simple modifications of the core isostere can significantly improve its potency. We hope that the potency of the α-keto amide **2** can be improved further by introduction of additional complementary groups to the proline ring moiety as illustrated in Figure 2. Computer modeling (Insight/Discover) indicates that attachment of hydrophobic groups to the proline ring moiety will enhance binding.

We have compared the activity of the isostere **2** to that of the identically substituted α-hydroxy amide precursors **17** and **18**. Consistent with observations by Sakurai[27] and with studies on

hydroxyethylamine dipeptide isosteres,[28,1] the *S*-diastereomer **18** (IC_{50} = 2μM) was found to be more potent than the *R*-diastereomer **17** (IC_{50} = 300 μM), but less active than **2**. The high potency of the α-hydroxy amide **18** implies that the hydroxyl group is hydrogen bonding more effectively with the catalytic carboxylic acid groups of HIV protease than in compound **17**, similar to that observed from the X-ray structure of the hydroxyethylamine inhibitor enzyme complex[29] (Figure 4). The stereochemistry of the isosteres **17** and **18** was determined by ^1H NMR studies on the *R*- and *S*-Mosher esters derived from the *S*-α-hydroxy ester **13**.[30, 31]

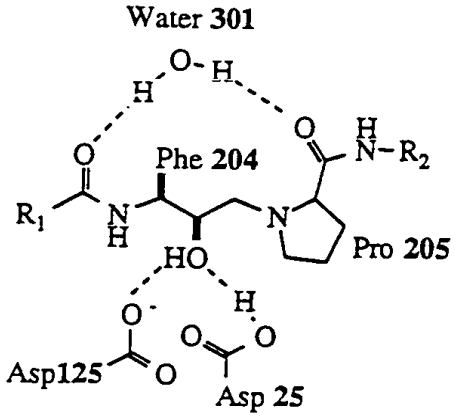
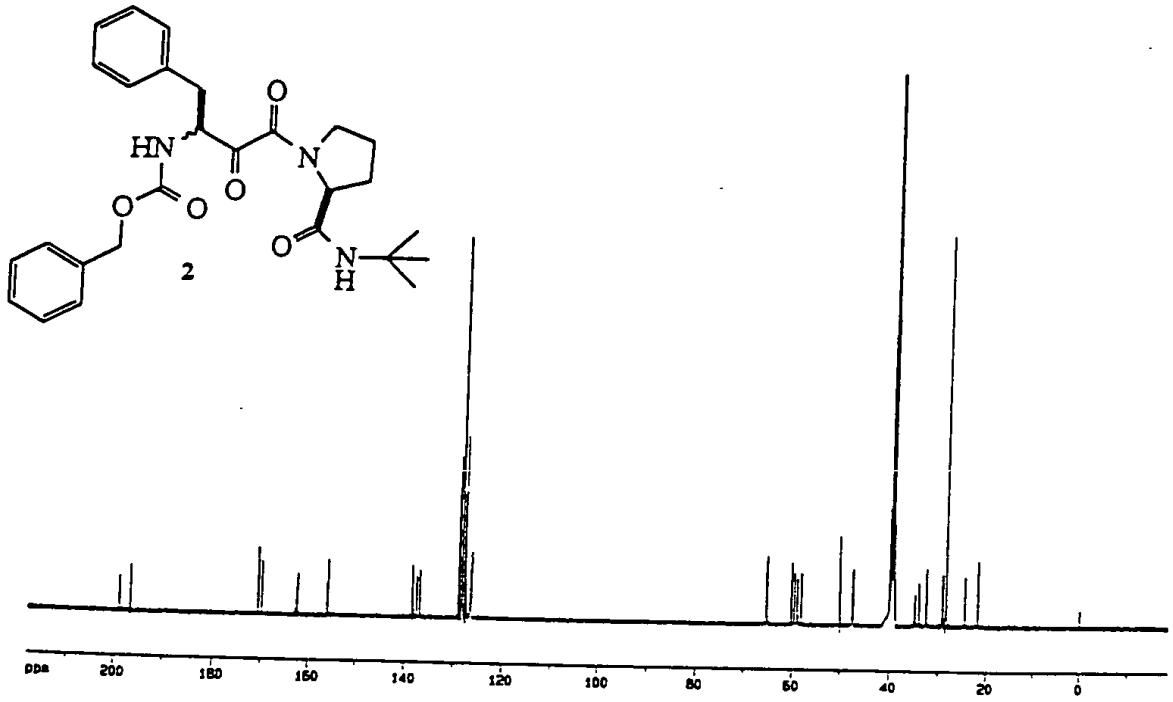
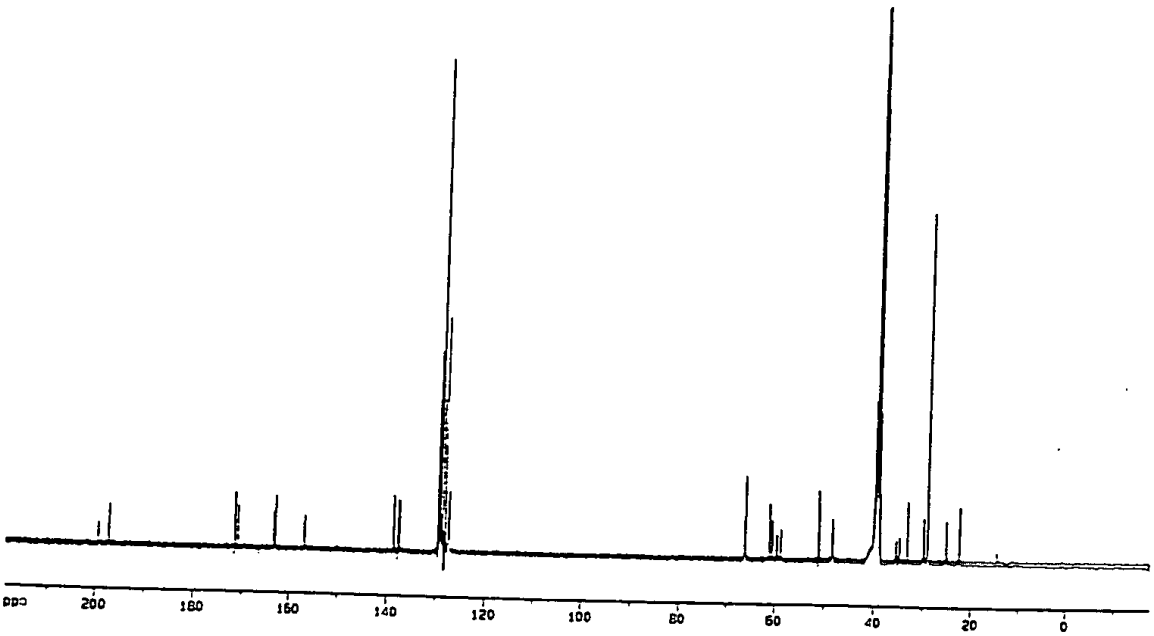


Figure 4. Schematic diagram illustrating hydrogen bond interactions between an hydroxyethylamine isostere and the active site of HIV protease, observed from the X-ray structure.

It is possible that the ketone moiety of the α-keto amide **2** is hydrated, as observed with other α-keto amides of this nature,[17,21] and is hydrogen bonding in a similar manner to that of compound **17**. However in this case, ^{13}C NMR studies on compound **2** in deuterated DMSO/D_2O (5:1) following the procedure described by Ocain and Rich[21] show that, in the presence of water, the ketone moiety of the α-keto amide *remains unhydrated* even after incubation for 24 hours (Figure 5). This would imply that the ketone moiety of **2** is quite stable in the presence of water and is therefore difficult to hydrate in the absence of a catalyst. It is likely that hydration of the ketone moiety takes place within the active site of HIV protease as illustrated in Figure 6, and the resulting hydrate is then stabilized through hydrogen bonding interactions with the aspartate residues of the enzyme. The hydrated form of **2** is considered to be a good transition state mimic.



^{13}C spectra of compound 2 in deuterated DMSO



^{13}C spectra in deuterated DMSO (500µl) and with D_2O (100µl) after incubation for 24 hours

Figure 5..

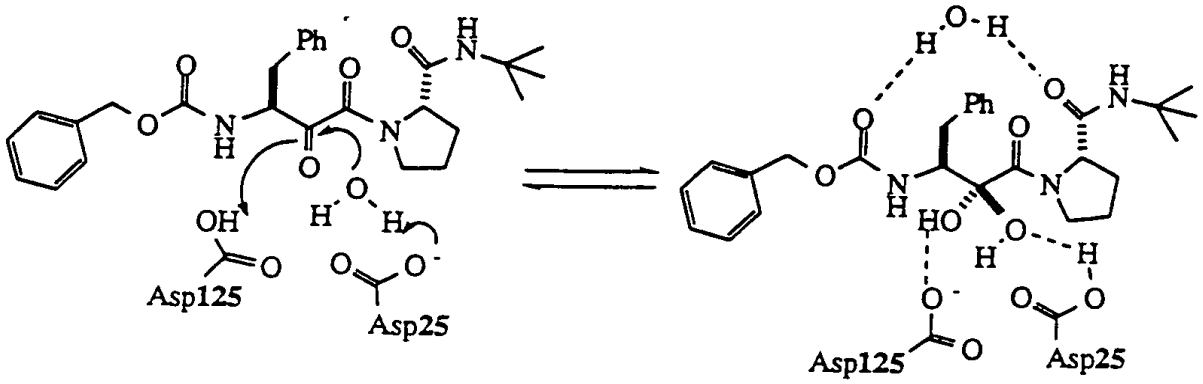


Figure 6. Schematic representation of general acid - general base mechanism for inhibitor **2** interaction with HIV protease aspartate groups

It is also possible, that the isostere **2** is interacting with HIV protease through a covalent mechanism (Figure 7) forming a hemiketal, in the same manner in which α-keto amides are thought to form hemithioketals with cysteine proteases,[32] or that **2** is bound to the enzyme non-covalently without any modification.

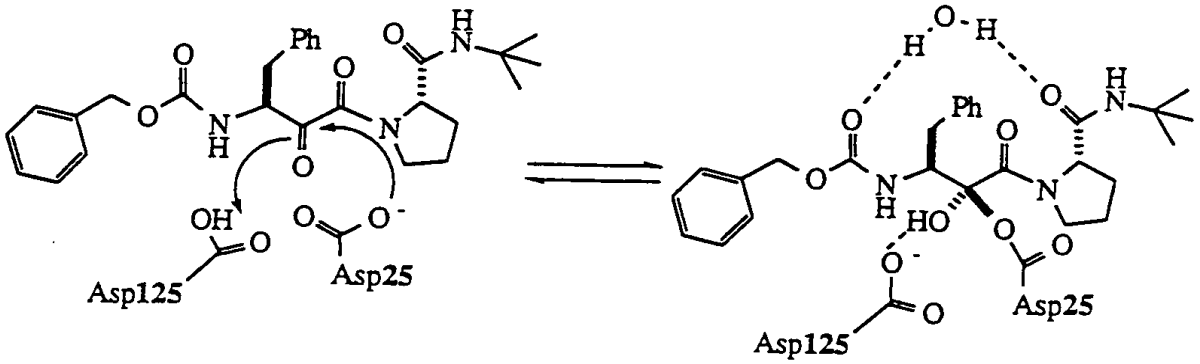


Figure 7. Schematic representation of nucleophilic mechanism for inhibitor **2** interaction with HIV protease aspartate groups

Time dependent assays do not exhibit time dependent inhibition. This implies that if the active form of the α-keto amide **2** is indeed the hydrate, the hydration step must be fast or **2** itself is the active form. Computer modeling and ion-spray mass spectrometry seem to indicate that the hydrated form is the active inhibitor (Olson, A.; Kent S. B. - details will be published separately). We are now in

the process of obtaining X-ray data of the complex between the α-keto amide **2** and HIV protease in order to clarify the mechanism of action.

When the α-keto amide **2** was tested against FIV protease, it was found to have no inhibitory effect when added in concentrations up to 70 mM. This result was surprising due to the similarity between the natural substrates for HIV and FIV proteases about the cleavage site as illustrated earlier. It would appear that FIV protease requires more specific residues within the P_4 - $P_{4'}$ sites than HIV protease, before it is able to recognize the core isostere **2** as a substrate. This is also supported by the observation that HIV protease will cleave an Acetyl-(6 residue) peptide substrate of sequence Gln-Ala-Tyr~Pro-Ile-Gln where as the smallest peptide FIV protease will cleave is an acetyl-(8 residue) peptide of sequence Pro-Gln-Ala-Tyr~Pro-Ile-Gln-Thr.[Kent unpublished,[33]] It is interesting to note that the corresponding protected dipeptide (Cbz-Phe-Pro-NBu^t) was not cleaved by HIV or FIVproteases under normal assay conditions.[34]

In an effort to develop inhibitors of the FIVprotease, we have found that the addition of suitable residues to interact with just the $P_{2'}$ and $P_{3'}$ sites of FIV protease is sufficient for moderate inhibition. Coupling of a side chain specific for FIV protease to the C-terminus of **2** gave **3** (Figure 8) as shown below. This extended isostere **3** was found to have an IC_{50} of 25μM and a K_i of 29 μM against FIV protease and the activity against HIV protease was slightly enhanced (K_i of 154 nM). It appears that the isosteric core structure of HIV protease inhibitors do not bind tightly to the FIV protease, and additional complementary groups are needed to enhance the binding. This difference is also observed in the analysis of other known HIV protease inhibitors. The potent cyclic urea based HIV protease inhibitor DMP 323 (IC_{50} = 36nM, K_i = 0.27nM)[35] for example was also found to be a very poor inhibitor of FIV protease (IC_{50} = 7.3mM). Perhaps further studies on the inhibition of these two enzymes and the resistant variants of HIV protease will provide some insights into the structure basis of drug resistance.

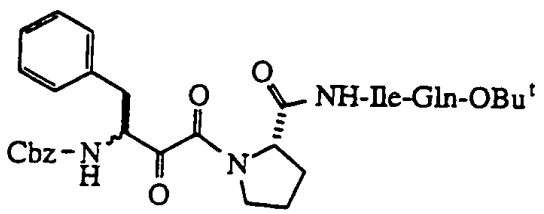


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Figure 8.

The preliminary results of our study have shown that the activity of the core hydroxyethylamine isostere **7** against HIV protease can be significantly enhanced by simple derivatization of the proline ring. The hydroxyethylamine derivatives shown in Figure 9 were synthesized in order to assess where favorable interactions about the proline ring could be made.

When the activity of the monomethylated derivative **6** is compared to that of the dimethylated derivative **4**, it can be seen that a hydrophobic moiety at the C-5 position decreases the potency of the isostere, whereas the activity increases when similar hydrophobic substitutions are made at the C-3 and C-4 positions as in compound **5**. The permethylated pyrrolidine derivative **5** was the most potent derivative of the series **4 - 6**, but significant overall activity was lost due to the absence of the amide bond at C-1, as can be seen by comparison of the activity of the methylated derivatives **4-6** to that of compound **7**. The C-4 substituted pyrrolidine derivative **8** was prepared, to confirm that an important favorable contribution to binding can be made by addition of a hydrophobic *cis* -C-4 substituent such as methoxy.

Work is in progress to prepare other C-4 substituted derivatives of **7** with an *N*-^{t}butyl amide moiety at C-1 in order to optimize interactions within the active site of HIV PR, and to investigate the activity of different ring sized 'proline' derivatives.

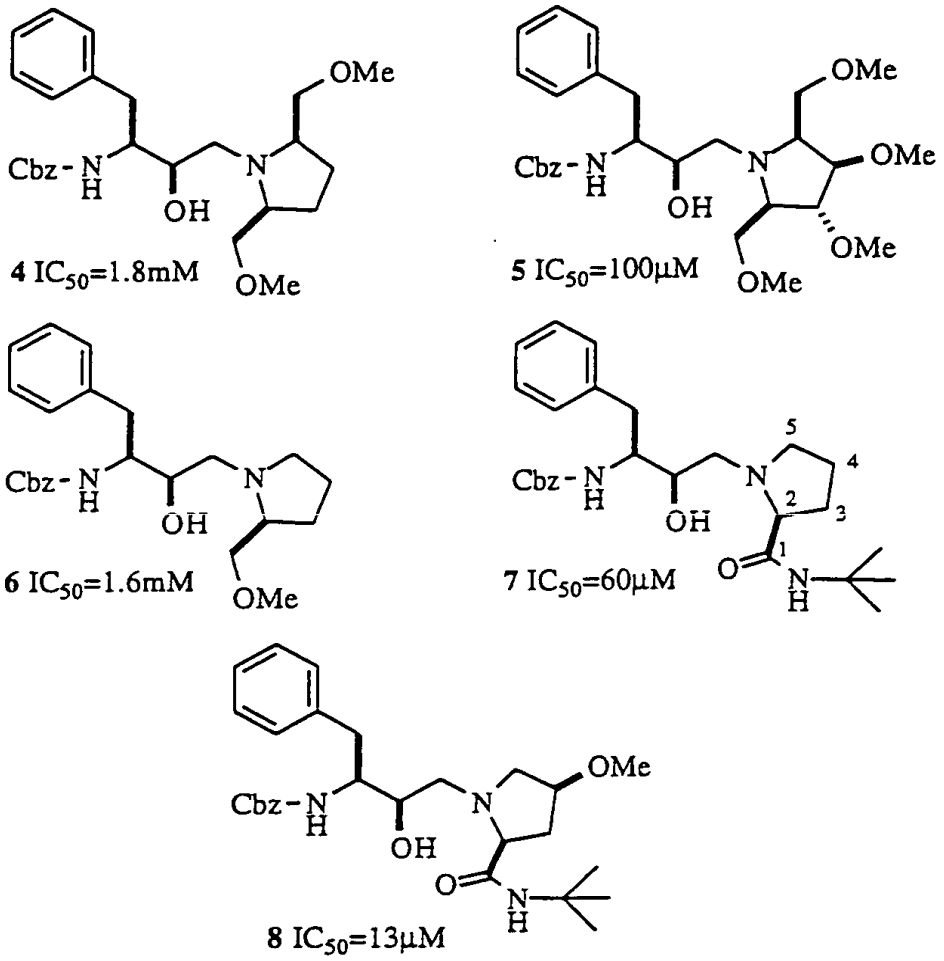
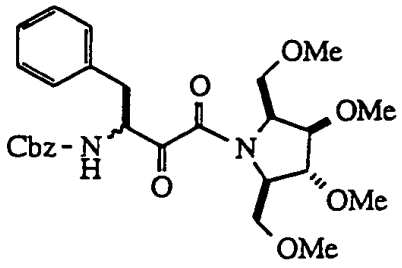


Figure 9. Inhibitory Activity of Variously Substituted Pyrrolidine Analogues against HIV Protease

The corresponding permethylated α-keto amide derivative **9** (Figure 10) was synthesized for comparison and was found to be significantly less potent against HIV PR (K_i = 20μM) than the original isostere **2**. This again illustrates the importance of an amide bond at C-1 of the pyrrolidine derivative.



9

Figure 10.

Table 1 summarizes the activities of the isosteres that we have tested against HIV PR and/or FIV PR.

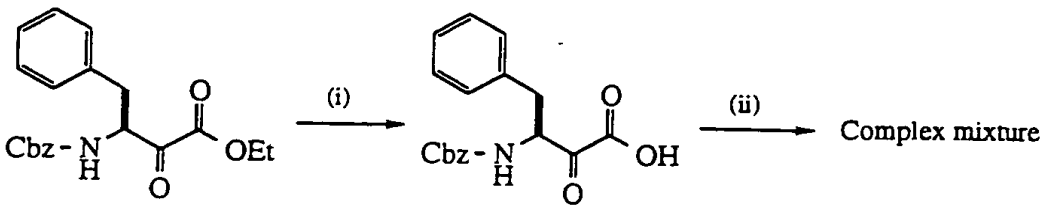
	HIV Protease		FIV protease	
Compound	IC_{50}	K_i	IC_{50}	K_i
1	6μM	6μM	-	-
2	700nM	214nM	>70mM	-
3	-	154nM	25μM	29μM
4	1.8mM	-	-	-
5	100μM	-	-	-
6	1.6mM	-	-	-
7	60μM	-	-	-
8	13μM	-	-	-
9	20μM	-	-	-

Table 1. Inhibition activities against HIV and/or FIV protease.

Chemistry

The synthesis of the core isostere **2** has been modified from the method previously employed by our group. The α-keto ester **10** was synthesized according to the method described by Angelastro.[36] It was not possible to cleanly couple the proline derivative **16** to the α-keto acid **11**, as multiple side

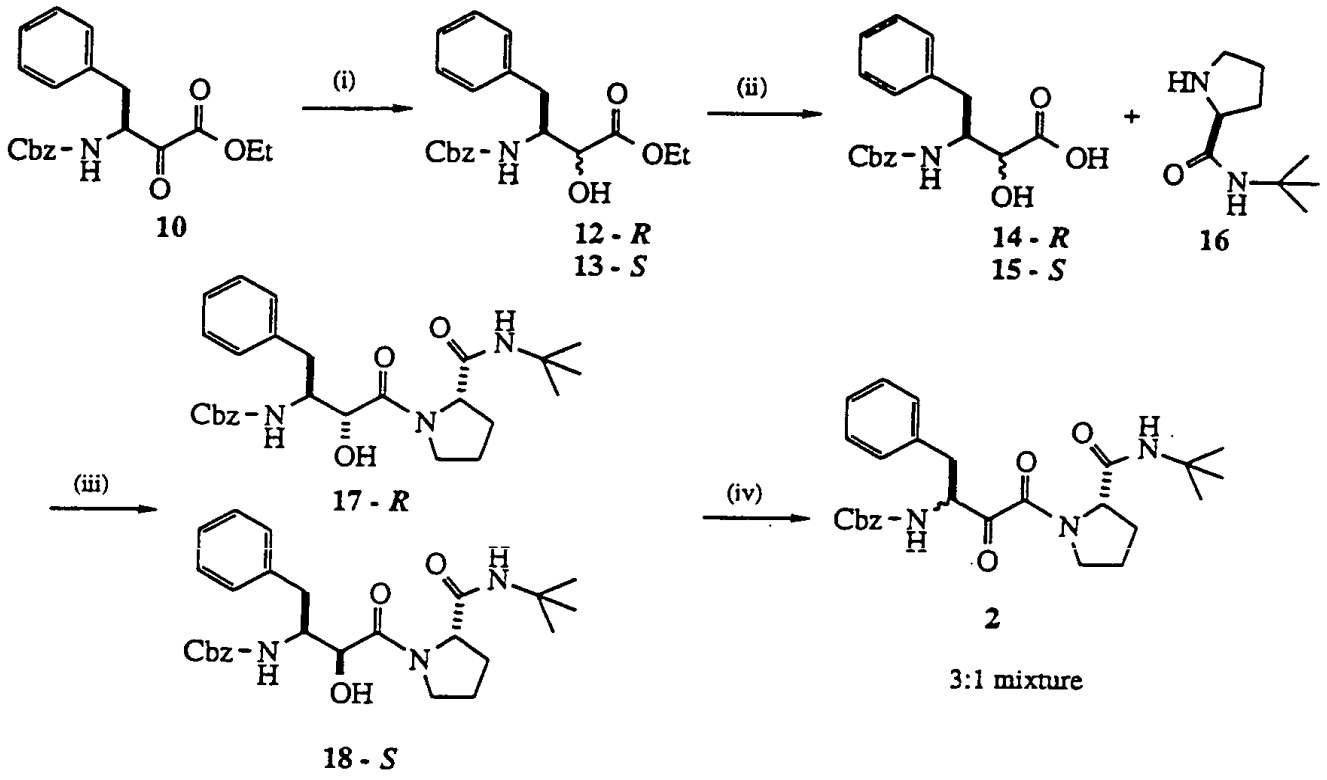
products were produced as a result of nucleophilic attack at the electrophilic ketone moiety (Scheme 1). All attempts at forming the dithiane or dithiolane derivative of the ketone moiety of **10**, resulted in low yields after tediously long reaction times, making this approach unfavorable.



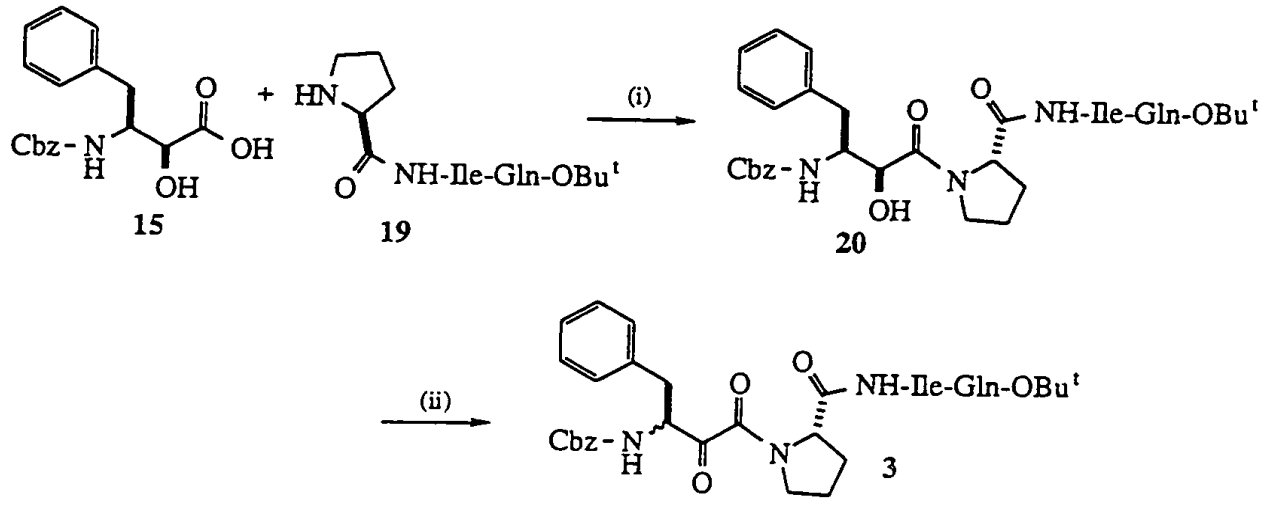
10 **11**

Scheme 1. (i) 0.17N LiOH, MeOH/H_2O, 2:1, 98%; (ii) **16**, EDC, HOBT, DIEA, DMF/CH_3CN, (1:1).

Reduction of the ketone moiety with sodium borohydride gave a separable mixture of diastereomeric alcohols **12** and **13** which could then be coupled to the desired proline derivatives after hydrolysis of the ester group. Hydrolysis of the ester moiety of **12** or **13** to the corresponding acids **14** or **15** respectively, followed by coupling to the proline derivative **16** gave the α-hydroxy amides **17** or **18**. Dess-Martin oxidation of either α-hydroxy amide **17** or **18** gave the desired α-keto amide **2** as a 3:1 mixture of diastereomers (Scheme 2).[37] When the α-keto amide **2** is dissolved in either methanol or DMSO the ratio of isomers changes immediately from 3:1 to 1:1. Racemization is due to the increased acidic nature of the hydrogen atom α- to the ketone moiety in **2** and has been observed with other similar α-keto amides.[20]



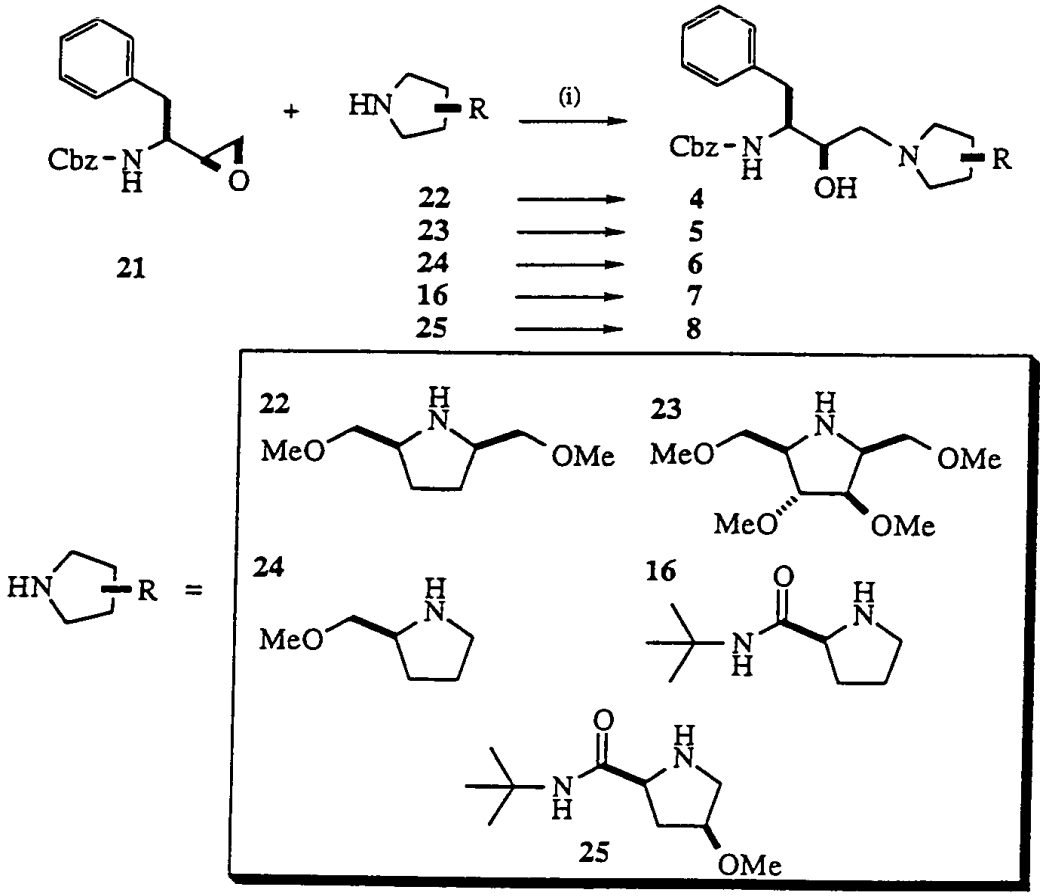
Scheme 2 (i) $NaBH_4$, MeOH, 0 °C, 95%; (ii) 0.17N LiOH, MeOH/H_2O, 2:1, 98%; (iii) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1), 84%; (iv) Dess-Martin periodinane, CH_2Cl_2, 95%.



Scheme 3. (i) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1), 80%; (ii) Dess-Martin periodinane, CH_2Cl_2, quantitative.

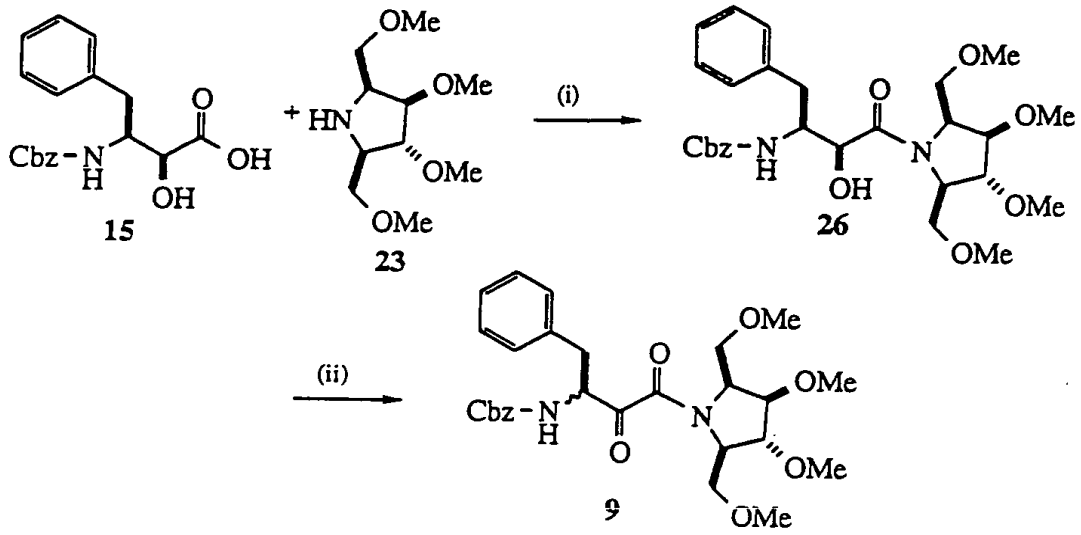
The extended α-keto amide isostere **3** used to inhibit FIV protease was synthesized in a similar manner to the original dipeptide isostere **2** with the only difference being that the *S*-α-hydroxy acid was coupled to the tripeptide **19** before oxidation to the desired α-keto acid **3** as illustrated in scheme 3.

The hydroxyethylamine inhibitors (compounds **4** - **8**) were prepared by coupling the desired pyrrolidine derivative to the epoxide **21**[38] *via* reflux in methanol, using triethylamine as shown in Scheme 4.



Scheme 4. (i) Methanol, Et_3N, reflux, 24 hours, 40-60%.

The permethylated α-ketoamide derivative **9** was prepared using the normal procedure used for the synthesis of α-keto amides as shown in Scheme 5.



Scheme 5. (i) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1), 80%; (ii) Dess-Martin periodinane, CH_2Cl_2, quantitative.

Acknowledgement. This work was supported by the NIH (GM48870). We wish to thank S. B. Kent, S. Walker for provision of the HIV protease.

Experimental Section

Biological Assays

For determination of IC_{50} values for HIV protease, backbone engineered HIV-1 protease, prepared by total chemical synthesis[39] 450 nM final concentration was added to a solution (152 µL final volume) containing inhibitor, 28 µM fluorogenic peptide substrate (sequence Abz-Thr-Ile-Nle-Phe-(*p*-NO_2)-Gln-Arg-NH_2)[34] and 1.8 % dimethylsulfoxide in assay buffer: 100mM MES buffer containing 0.5 mg/cm^3 BSA (Bovine Serum Album, fatty acid, nuclease and protease free - to stabilize enzyme) at pH 5.5. The solution was mixed and incubated over 5 minutes during which time the rate of substrate cleavage was monitored by continuously recording the change in fluorescence of the assay solution. An excitation filter of 325 nm, and an emission filter of 420nm was used. This data was converted into µM substrate cleaved per minute, using a predetermined standard calibration curve of change in fluorescence against concentration of substrate cleaved.

Determination of K_i for HIV protease was performed similarly with the following modifications. The substrate concentrations used were 57, 43, 28 and 14 μM. All other concentrations were as above. The curve fit for the data was determined and the subsequent K_i derived using a computer program based on the equation of Morrison [40] for tight binding inhibitors.

For determination of K_i and IC_{50} for FIV protease, the enzyme 0.125 μg was added to a solution (100μL final volume) containing inhibitor, 560 μM peptide substrate (sequence Gly-Lys-Glu-Glu-Gly-Pro-Pro-Gln-Ala-Tyr~Pro-Ile-Gln-Thr-Val-Asn-Gly) and 2% dimethyl sulfoxide in a 1:3 mixture of assay buffer (as above) and 4M $NaCl_{aq.}$ solution. The solution was mixed and incubated for 10 minutes at 37 °C and the reaction quenched by addition of 8M guanidine HCl solution containing 0.2 M sodium acetate at pH 4.2 (100 μL). The cleavage products and substrate were separated by reverse phase HPLC. Absorbance was measured at 215 nm, peak areas were determined and percent conversion to product was calculated using relative peak areas. The data were plotted as 1/V (V = rate substrate is cleaved in nmol/min) against inhibitor concentration and the $-K_i$ determined as the point at which the resulting line intersects with $1/V_{max}$ (V_{max} = 6.85 nmol/min). IC_{50} was determined as the inhibitor concentration at 50% inhibition. V_{max} (6.85 ± 0.7 nmol min^{-1}) and K_m (707 ±70μM) for FIV protease were determined from a plot of 1/V (V = rate in nmol/min) against 1/[S] ([S] = substrate concentration in nmol). The data used was generated similarly to that for K_i with the following modifications. The substrate concentrations used were 560, 448, 336, 224, 111 and 56 μM, in the absence of inhibitor.

Purification of FIV PR:

A 503 base pair Eco R1-Bam H1 fragment containing the coding sequence of FIV protease was cloned from FIV-34TF10[41] into the PT7-7 vector.[42] The 5' end of the insert was modified by the addition of an Nde1 adaptor, which provided the proper reading frame with initiation of translation from the methionine encoded in the latter site. The construct was transformed into *E. coli* strain BL21.DE3, lys S[43] and overnight cultures were used to inoculate 15 liter fermentations, performed

using Circlegrow medium (Bio 101) plus 100 μL ampicillin, 20 μM chloramphenicol, at 37 °C. The cells were allowed to reach mid-log phase, then the temperature was reduced to 24 °C and IPTG was added to a final concentration of 1 mM. The fermentation was allowed to proceed for 16h, at which time the cells were harvested by centrifugation and frozen at -70 °C in 100 g aliquots for future use.

Cells (100 g) were lysed by addition of 600 cm^3, 50 mM Tris-HCl, pH 8, 5 mM EDTA and 2 mM 2-mercaptoethanol to the frozen pellet. The cells lysed upon thawing and the viscous mixture was homogenized at 4 °C for 2 min in a Waring blender. The sample was centrifuged at 8,000 x g for 20 min and the pellet discarded. The sample was diluted to 1 liter, then subjected to tangential flow against a 300 K cut-off membrane (Filtron) and the PR was washed through the membrane using five liters of the same buffer. The retentate was discarded and the flow-through supernatant concentrated by tangential flow against a 10 K cut-off membrane. The retentate was passed over a DE52 anion exchange column (5 x 20 cm) equilibrated in the same buffer. The flow-through from this column was passed over an S-Sepharose Fast Flow matrix (2.5 x 20 cm column, Pharmacia), again equilibrated at pH 8 in the same buffer. The flow-through from S-Sepharose was made 1M with respect to ammonium sulfate and applied to a phenyl sepharose column (Pharmacia, 1.5 x 10 cm), washed with lysis buffer containing 1M ammonium sulfate, then eluted with a 100-0% linear ammonium sulfate gradient. Peak fractions containing PR were pooled, concentrated using Centripreps (Amicon), and dialyzed against 10 mM TrispHCl, pH 8, 5 mM EDTA, 2 mM 2-mercaptoethanol. The sample was made 10 mM with respect to MOPS, adjusted to pH 5.5 with HCl, then applied to a Resource S column (Pharmacia) equilibrated in 10 mM Tris-MOPS, pH 5.5, 5 mM EDTA and 2 mM 2-mercaptoethanol. PR was eluted using a linear 0-300 mM NaCl gradient in the same buffer. Peak fractions were pooled, concentrated, and stored as aliquots at -20 °C for further studies. The isolated FIV PR was confirmed by ion spray mass spectrometry.

Chemical Synthesis

General procedures. All manipulations were conducted under an inert atmosphere (argon or nitrogen). All solvents were reagent grade. Anhydrous ether, tetrahydrofuran (THF), and toluene

were distilled from sodium and/or benzophenone ketyl. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride (CaH_2). *N*, *N*, Dimethylformamide (DMF) and acetonitrile were distilled from phosphorous pentoxide and calcium hydride. Methanol was distilled from magnesium and iodine. Organic acids and bases were reagent grade. All other reagents were commercial compounds of the highest purity available. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel (60 F-254) plates (0.25 mm). Visualization was effected using standard procedures unless otherwise stated. Flash column chromatography was carried out on Merck silica gel 60 particle size (0.040-0.063 mm, 230-400 Mesh). Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton and carbon magnetic resonance spectra (^1H-, ^{13}C-NMR) were recorded on either a Bruker AM-500, AMX-400 or AC250MHz Fourier transform spectrometer. Coupling constants (*J*) are reported in hertz and chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS, 0 ppm), MeOH (3.30 ppm for ^1H and 49.0 ppm for ^{13}C) or $CHCl_3$ (7.24 ppm for ^1H and 77.0 ppm for ^{13}C) as internal reference. Infrared spectra (IR) were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer. Absorptions are reported in wavenumbers (cm^{-1}).

Peptide fragments described herein were synthesized using traditional peptide coupling methodologies [EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl), HOBt (1-hydroxybenzotriazole) and DIEA (diisopropylethylamine)]. Esters were hydrolyzed either by base (LiOH for methyl esters) or acid (TFA for ^{t}-butyl esters).

(2*R*, 3*S*) and (2*S*, 3*S*) *N*-(Benzyloxycarbonyl)-AHAP-(3-Amino-2-Hydroxy-4-Phenylbutanoic Acid) Ethyl Ester 12 and 13 respectively

The substrate **10** (600 mg, 1.7 mmol) was dissolved in methanol (10 cm^3) and cooled to 0 °C. Sodium Borohydride (70.3 mg, 1.9 mmol) was then added. After 20 minutes the reaction was quenched by addition of saturated ammonium $chloride_{(aq.)}$ (10 cm^3). The reaction mixture was concentrated *in vacuo* to remove most of the methanol. The aqueous residue was then extracted with ethyl acetate (3 x 20 cm^3), washed with brine (10 cm^3), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a mixture of diastereomers. The alcohols were separated by flash chromatography eluting with 15% ethyl acetate in hexane to give the alcohols in a ratio of 3:4, **12** & **13** (590 mg, 97%). R_f = 0.54 and 0.40 respectively (EtOAc/Hexane, 1:2):

12 (2*R*, 3*S* - colorless oil); ^1H NMR (500MHz, $CDCl_3$) δ 7.34-7.18 (10H, m), 5.17 (1H, d, *J* 9.5), 5.04 (2H, s), 4.43-4.38 (1H, m), 4.33 (1H, dd, *J* 4.5, 2.0), 4.15-4.08 (1H, m), 3.98-3.92 (1H, m), 3.28 (1H, d, *J* 5.0); IR (NaCl) υ_{max} 3368, 3030, 2981, 1731, 1520, 1455, 1246, 1104, 1055, 748, 699 cm^{-1}; FABHRMS (NBA) *m/e* 358.1659 ($[M+H]^+$, $C_{20}H_{23}NO_5$ requires 358.1654); (Found: C, 67.30; H, 6.50; N, 3.99. $C_{20}H_{23}NO_5$ requires C, 67.21; H, 6.49; N, 3.92%).

13 (2*S*, 3*S* - crystalline); ^1H NMR (500MHz, $CDCl_3$) δ 7.34-7.18 (10H, m), 5.17 (1H, d, *J* 9.5), 5.04 (2H, s), 4.43-4.38 (1H, m), 4.33 (1H, dd, *J* 4.5, 2.0), 4.15-4.08 (1H, m), 3.98-3.92 (1H, m), 3.28 (1H, d, *J* 5.0); IR (NaCl) υ_{max} 3368, 3030, 2980, 1731, 1520, 1455, 1246, 1104, 1055, 748, 699 cm^{-1}; FABHRMS (NBA) *m/e* 358.1661 ($(M^+ + H)$, $C_{20}H_{23}NO_5$ requires 358.1654); (Found: C, 67.22; H, 6.57; N, 3.90. $C_{20}H_{23}NO_5$ requires C, 67.21; H, 6.49; N, 3.92%). m.p. 88-89°C.

(2*R*, 3*S*) and (2*S*, 3*S*) *N*-(Benzyloxycarbonyl)-3-Amino-2-Hydroxy-4-Phenylbutanoic Acid 14 & 15 respectively

The substrate (**12** or **13**) (250 mg, 0.70 mmol) was dissolved in 0.25 N LiOH in methanol/water, 2:1 (5 cm^3), and stirred at ambient temperature for 30 minutes. The pH of the reaction was adjusted to pH 7.0 with 1N $HCl_{(aq.)}$ and the methanol removed *in vacuo*. The aqueous residue was then acidified to pH 2.0 with 1N $HCl_{(aq.)}$ and extracted with ethyl acetate (3 x 30 cm^3). The combined organic extracts were washed with water (10 cm^3), brine (10 cm^3) and dried ($MgSO_4$) before concentration *in vacuo* to give the desired acid (**14** or **15**) as a white solid (212 mg, 92%). The acids were purified by recrystallization from hot ethanol.

14 (2*R*, 3*S*); ^1H NMR (500MHz, CD_3OD) δ 7.31-7.18 (10H, m), 6.87 (1H, d, *J* 9.5), 5.00 (1H, d, *J* 10.0), 4.96 (1H, d, *J* 10.0), 4.31-4.23 (1H, m), 4.07 (1H, d, *J* 2.5), 2.93 (1H, dd, *J* 13.5, 7.5), 2.83 (1H, dd, *J* 13.5, 8.0); ^{13}C NMR (125MHz, CD_3OD) δ 176.6 (C=O), 158.5 (C=O), 139.8 (C), 138.5 (C), 130.7 (2 x CH), 129.8 (2 x CH), 129.7 (2 x CH), 129.7 (CH), 129.1 (CH), 128.8 (CH), 127.8 (CH), 67.6 (CH), 57.1 (CH_2), 57.0 (CH_2), 39.3 (CH); FABHRMS (NBA) *m/e* 330.1353 ($[M+H]^+$, $C_{18}H_{19}NO_5$ requires 330.1341); m.p. 209-210 (decomp.).

15 (2*S*, 3*S*); ^1H NMR (500MHz, CD_3OD) δ 7.28-7.18 (10H, m), 7.09 (1H, d, *J* 12.5), 4.97 (1H, d, *J* 12.5), 4.92 (1H, d, *J* 12.5), 4.26 (1H, d, *J* 4.0), 4.25-4.20 (1H, m), 2.81 (1H, dd, *J* 14.0, 4.0), 2.76 (1H, dd, *J* 14.0, 4.0); ^{13}C NMR (125MHz, CD_3OD) δ 175.9 (C=O), 158.5 (C=O), 140.0 (C), 138.6 (C), 130.6 (3 x CH), 129.6 (CH), 129.5 (3 x CH), 129.0 (CH), 128.8 (CH), 127.6 (CH), 74.3 (CH), 67.4 (CH_2), 57.1 (CH), 36.5 (CH_2); FABHRMS (NBA/NaI) *m/e* 352.1174 ($[M+Na]^+$, $C_{18}H_{19}NO_5$ requires 352.1161); (Found: C, 65.34; H, 5.75; N, 4.33. $C_{18}H_{19}NO_5$ requires C, 65.64; H, 5.82; N, 4.25%); m.p. 173-174°C (decomp.).

N-(*tert*-Butoxycarbonyl)-L-prolyl-*tert*-butyl amide

The substrate *N-tert*-Butoxycarbonyl-L-proline (3.0 g, 13.9 mmol) was dissolved in dry CH_2Cl_2 (20 cm^3). HOBT (2.07 g, 15.3 mmol), EDC (2.93 g, 15.3 mmol), and *tert*-butylamine (1.6 cm^3, 15.3 mmol), were added and the mixture stirred for 18 hours at ambient temperature. The reaction was diluted with ethyl acetate (100 cm^3), and washed with water (2 x 20 cm^3), 1 N $HCl_{(aq.)}$ (10 cm^3), saturated sodium bicarbonate solution $_{(aq.)}$ (10 cm^3), water (10 cm^3), brine (10 cm^3) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Purification by flash chromatography, eluting with 33% EtOAc in Hexane gave *N-tert*-butoxycarbonyl-L-prolyl-*tert*-butyl amide as a colorless oil (1.53 mg, 40%). $R_f = 0.46$ (EtOAc/Hexane, 1:1).

^1H NMR signals broadened due to rotamers:

^1H NMR (500MHz, $CDCl_3$) δ 7.31-7.25 (5H, m), 6.35 (1H, br s), 4.60-4.15 (3H, m), 3.55-3.22 (2H, m), 2.41-1.70 (4H, m), 1.60-1.18 (9H, br s); IR (NaCl) υ_{max} 3298, 3086, 2976, 1698, 1660, 1531, 1398, 1162 cm^{-1}; FABHRMS (NBA/NaI) *m/e* 293.1832 ($[M+Na]^+$, $C_{14}H_{26}N_2O_3$ requires 293.1841).

L-Prolyl-*tert*-butyl amide 16.

N-tert-Butoxycarbonyl-L-prolyl-*tert*-butyl amide (600 mg, 2.22 mmol) was dissolved in CH_2Cl_2 (10 cm^3) and cooled to 0 °C. TFA (10 cm^3) was then added to the solution. After 1 hour at 0 °C the reaction was concentrated *in vacuo* (any remaining TFA was removed under high vacuum) to give the trifluoroacetic acid salt of the desired amine **16** as a colorless oil (800 mg, 95%). The amine was used without further purification in subsequent coupling steps.

^1H NMR (500MHz, $CDCl_3$) δ 7.30 (1H, br s), 6.90 (1H, br s), 4.52 (1H, br s), 3.35 (2H, br s), 2.49-2.34 (1H, m), 2.08-1.97 (3H, m), 1.34 (9H, s); ^{13}C NMR (125MHz, $CDCl_3$) δ 167.4 (C=O), 59.6 (CH), 52.3 (C), 46.6 (CH_2), 30.4 (CH_2), 28.2 (3 x CH_3), 24.6 (CH_2); FABHRMS (NBA) *m/e* 171.1500 ($[M+H]^+$, $C_9H_{18}N_2O$ requires 171.1497).

General peptide coupling procedure:

(2 *S*, 3*R*) and (2*S*, 3*S*) 3-(*N*-Benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-L-prolyl-*tert*-butyl amide 17 and 18.

The substrate **14** or **15** (70 mg, 0.213 mmol), was dissolved in dry DMF (3 cm^3). HOBT (31 mg, 0.22 mmol), EDC (43 mg, 0.224 mmol), DIEA (122 µl, 0.703 mmol) were added and the mixture stirred for 30 minutes at room temperature. The secondary amine **16** as its TFA salt (73 mg, 0.255 mmol) was added and the reaction stirred for 18 hours. The reaction mixture was diluted with ethyl acetate (20 cm^3) and added to saturated ammonium chloride (30 cm^3). The aqueous phase was extracted with ethyl acetate (3 x 10 cm^3). The combined organic phases were then washed with water (2 x 5 cm^3), 1 N $HCl_{(aq.)}$ (5 cm^3), saturated sodium bicarbonate solution $_{(aq.)}$ (50 cm^3), water (5 cm^3), brine (5 cm^3) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography eluting with ethyl acetate/hexane, 1:1 to give the desired coupled product **17** or **18** as a colorless oil (74 mg, 72%). R_f = 0.33 and 0.28 respectively (EtOAc:Hexane, 1:1).

17 (2*R*, 3*S*); ^1H NMR (500MHz, $CDCl_3$) δ 7.37-7.23 (10H, m), 6.44 (1H, s), 5.16 (1H, d, *J* 9.5), 5.03 (2H, s), 4.24-4.18 (1H, m), 4.11 (1H, d, *J* 5.5), 3.97-3.90 (2H, m), 3.28-3.23 (1H, m), 3.12-3.06 (1H, m), 2.99-2.90 (2H, m), 2.18-2.12 (1H, m), 2.01-1.96 (1H, m), 1.89-1.83 (1H, m), 1.83-1.76 (1H, m), 1.27 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 171.4 (C=O), 169.9 (C=O), 156.0 (C=O), 137.4 (C), 136.7 (C), 129.2 (2 x CH), 128.7 (2 x CH), 128.4 (2 x CH), 128.0 (CH), 127.9 (2 x CH), 126.9 (CH), 68.6 (CH), 66.7 (CH_2), 61.7 (CH), 52.8 (CH), 51.1 (C), 46.1 (CH_2), 38.4 (CH_2), 28.6 (3 x CH_3), 27.4 (CH_2), 24.7 (CH_2); IR (NaCl) υ_{max} 3318, 2966, 1714, 1667, 1537, 1454, 1366, 1041 cm^{-1}; FABHRMS (NBA) *m/e* 482.2677 ($[M+H]^+$, $C_{27}H_{35}N_3O_5$ requires 482.2655); (Found: C, 67.22; H, 7.32; N, 8.80. $C_{27}H_{35}N_3O_5$ requires C, 67.34; H, 7.33; N, 8.73%).

18 (2*S*, 3*S*); (major rotamer only) ^1H NMR (400MHz, $CDCl_3$) δ 7.33-7.17 (10H, m), 6.45 (1H, s), 5.21 (1H, d, *J* 8.8), 4.99 (2H, s), 4.60 (1H, dd, *J* 6.8, 2.1), 4.52-4.47 (1H, m), 4.21-4.13 (1H, m), 3.81-3.66 (3H, m), 2.75-2.60 (2H, m), 2.41-2.30 (1H, m), 2.25-2.09 (1H, m), 2.05-1.90 (2H, m),

1.30 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 171.3 (C=O), 169.6 (C=O), 156.0 (C=O), 137.3 (2 x C), 129.1 (2 x CH), 128.5 (2 x CH), 128.4 (2 x CH), 128.0 (CH), 127.8 (2 x CH), 126.5 (CH), 71.3 (CH), 66.6 (CH_2), 61.1 (CH), 54.3 (CH), 51.3 (C), 47.5 (CH_2), 33.8 (CH_2), 28.6 (3 x CH_3), 26.9 (CH_2), 25.4 (CH_2); IR (NaCl) υ_{max} 3318, 2966, 1714, 1667, 1537, 1454, 1366, 1041 cm^{-1}; FABHRMS (NBA/CsI) *m/e* 614.1645 ($[M+Cs]^+$, $C_{27}H_{35}N_3O_5$ requires 614.1631); (Found: C, 67.20; H, 7.66; N, 8.54. $C_{27}H_{35}N_3O_5$ requires C, 67.34; H, 7.33; N, 8.73%).

General Dess-Martin oxidation procedure:

(3S) and (3*R*) 3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-L-prolyl-*tert*-butyl amide 2.

The substrate **17** (21 mg, 0.044 mmol) was dissolved in dry CH_2Cl_2 (2 cm^3), and Dess-Martin periodinane (26 mg, 0.088 mmol) added. The reaction mixture was stirred at ambient temperature for 24 hours, then diluted with ethyl acetate (10 cm^3) and quenched by addition of saturated sodium bicarbonate $_{(aq.)}$ (5 cm^3) and sodium thiosulfate. The aqueous phase was extracted with ethyl acetate (3 x 20 cm^3). The combined organic extracts washed with water (10 cm^3), brine (10 cm^3), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gave the desired product **2** as a 3:1 mixture of diastereomers (colorless oil) (20 mg, 95%). R_f = 0.47 (EtOAc/Hexane, 1:2).

Spectral data on mixture:

^1H NMR (400MHz, DMSO) δ 7.86 (1H, d, *J* 7.8), 7.71 (1H, d, *J* 8.3), 7.64 (1H, s), 7.53 (1H, s), 7.37-7.10 (20 H, m), 5.10 (1H, ddd, *J* 11.0, 8.3, 2.4), 5.01 (1H, d, *J* 12.6), 4.95 (1H, d, *J* 12.6), 4.95 (1H, d, *J* 16.3), 4.88 (1H, d, *J* 16.3), 4.79-4.73 (1H, m), 4.66 (1H, dd, *J* 7.8, 4.2), 4.26 (1H, dd, *J* 7.8, 4.5), 3.60-3.37 (3H, m), 3.33-3.24 (1H, m), 3.18 (1H, dd, *J* 14.7, 2.4), 3.13 (1H, dd, *J* 10.2, 3.9), 2.79 (1H, dd, *J* 13.7, 10.2), 2.46 (1H, dd, *J* 14.7, 11.0), 2.23-2.17 (1H, m), 2.04-1.97 (1H, m), 1.90-1.60 (6H, m), 1.24 (9H, s), 1.22 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 198.91 (C=O), 196.7 (C=O), 170.7 (C=O), 169.9 (C=O), 162.6 (C=O), 162.2 (C=O), 156.1 (C=O), 155.9 (C=O), 138.5 (C), 137.6 (C), 136.9 (C), 136.9 (C), 129.0 (CH x 2), 128.8 (CH x 2), 128.4 (CH x 8), 127.8 (CH x

2), 127.6 (CH x 2), 127.6 (CH x 2), 126.5 (CH), 126.4 (CH), 65.6 (CH_2), 65.3 (CH_2), 60.3 (CH), 59.7 (CH), 59.2 (CH), 58.2 (CH), 50.3 (C), 50.1 (C), 47.6 (CH_2), 47.4 (CH_2), 34.8 (CH_2), 34.1 (CH_2), 32.5 (CH_2), 29.1 (CH_2), 28.5 (CH_3 x 3), 28.4 (CH_3 x 3), 24.5 (CH_2), 21.7 (CH_2); IR (NaCl) υ_{max} 3325, 2966, 1715, 1670, 1634, 1531, 1454, 1258, 1051, 738, 699; FABHRMS (NBA/NaI) *m/e* 502.2295 ($[M+Na]^+$, $C_{27}H_{33}N_3O_5$ requires 502.2318); (Found: C, 67.62; H, 7.05; N, 8.96. $C_{27}H_{33}N_3O_5$ requires C, 67.62; H, 6.94; N, 8.76%).

L-Prolyl-L-isoleucyl-L-glutamine-*tert*-butyl amide 19

Spectral data:

^1H NMR (400MHz, CD_3OD) δ 4.35-4.30 (1H, m), 4.31 (1H, dd, *J* 9.3, 5.0), 4.21 (1H, d, *J* 7.8), 3.41-3.26 (2H, m), 2.48-2.36 (1H, m), 2.36-2.22 (2H, m), 2.20-1.79 (6H, m), 1.67-1.54 (1H, m), 1.46 (9H, s), 1.32-1.17 (1H, m), 0.99 (3H, d, *J* 6.8), 0.93 (3H, t, *J* 7.4); C^{13} NMR (100MHz, CD_3OD) δ 173.3 (C=O), 172.0 (C=O), 170.1 (C=O), 167.7 (C=O), 83.0 (C), 60.7 (CH), 59.9 (CH), 54.0 (CH), 47.4 (CH_2), 37.9 (CH), 32.5 (CH_2), 31.2 (CH_2), 28.4 (CH_2), 28.2 (3 x CH_3), 26.0 (CH_2), 25.0 (CH_2), 15.9 (CH_3), 11.4 (CH_3); FABHRMS (NBA) *m/e* 435.2575 ($[M+Na]^+$, $C_{20}H_{36}N_4O_5$ requires 435.2583).

(2*S*, 3*S*) 3-(*N*-Benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-L-prolyl-L-isoleucyl-L-glutamine-*tert*-butyl amide 20

The coupling of the acid **15** to the amide **19** was carried out using the general peptide coupling procedure. Flash chromatography eluting with 5% methanol in dichloromethane gave the desired product **19** as a colorless oil (45 mg, 80%). $R_f = 0.24$ (5% MeOH in CH_2Cl_2).

^1H NMR (400MHz, $CDCl_3$) (major rotamer only) δ 7.42-7.12 (11H, m), 6.95 (1H, d, *J* 8.6), 6.79 (1H, s), 6.07 (1H, s), 5.95 (1H, d, *J* 8.3), 5.03 (1H, d, *J* 12.4), 4.96 (1H, d, *J* 12.4), 4.65 (1H, d, *J* 4.5), 4.51 (1H, dd, *J* 8.0, 5.2), 4.49-4.35 (2H, m), 4.32 (1H, d, *J* 7.8), 3.99-3.94 (1H, m), 3.70-3.50

(2H, m), 2.91-2.83 (2H, m), 2.28-2.21 (1H, m), 2.21-2.10 (3H, m), 2.02-1.75 (5H, m), 1.43 (9H, s), 1.14-1.06 (1H, m), 0.86 (3H, d, *J* 6.4), 0.78 (3H, t, *J* 7.6); C^{13} NMR (100MHz, $CDCl_3$) δ 175.5 (C=O), 172.2 (C=O), 171.5 (2 x C=O), 170.4 (C=O), 156.3 (C=O), 137.9 (C), 136.3 (C), 129.1 (2 x CH), 128.4 (4 x CH), 128.0 (CH), 127.6 (2 x CH), 126.4 (CH), 82.4 (C), 71.4 (CH), 66.5 (CH_2), 61.2 (CH), 57.8 (CH), 55.5 (CH), 52.3 (CH), 47.7 (2 x CH_2), 37.0 (CH), 33.8 (CH_2), 31.48 (CH_2), 28.76 (CH_2), 27.9 (3 x CH_3), 25.3 (CH_2), 24.8 (CH_2), 15.3 (CH_3), 11.0 (CH_3); FABHRMS (NBA/CsI) *m/e* 856.288 ($[M+Cs]^+$, $C_{38}H_{53}N_5O_9$ requires 856.2898).

(3*S*) and (3*R*) 3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-L-prolyl-L-isoleucyl-L-glutamine-*tert*-butyl amide 3

Oxidation of **20** was carried out using the general Dess-Martin oxidation procedure outlined above. Purification by flash chromatography eluting with 5% methanol in dichloromethane gave the desired α-keto amide **3** (2:1 mixture of isomers) as a colorless oil (37 mg, quantitative). $R_f = 0.24$ (EtOAc).

(3*S*)

^1H NMR (400MHz, $CDCl_3$) δ 7.38-7.16 (11H, m), 6.98 (1H, d, *J* 8.5), 6.80 (1H, s), 6.64 (1H, s), 5.64 (1H, d, *J* 8.3), 5.09 (1H, d, *J* 12.2), 5.02 (1H, d, *J* 12.2), 4.46 (1H, dd, *J* 8.2, 3.4), 4.21 (1H, t, *J* 8.1), 3.67-3.60 (1H, m), 3.59-3.45 (1H, m), 3.70-3.50 (2H, m), 3.25 (1H, dd, *J* 14.1, 5.5), 3.09 (1H, dd, *J* 14.1, 8.5), 2.29-2.12 (4H, m), 2.12-1.78 (6H, m), 1.46 (9H, s), 1.20-1.05 (1H, m), 0.93-0.83 (6H, m, 2 x CH_3); C^{13} NMR (100MHz, $CDCl_3$) δ 197.1 (C=O), 175.1 (C=O), 171.1 (C=O), 170.5 (2 x C=O), 162.9 (C=O), 156.3 (C=O), 135.7 (2 x C), 129.1 (2 x CH), 128.7 (2 x CH), 128.5 (2 x CH), 128.5 (CH), 127.5 (2 x CH), 126.9 (CH), 82.4 (C), 77.2 (CH), 67.3 (CH_2), 61.0 (CH), 58.0 (CH), 52.3 (CH), 48.0 (2 x CH_2), 37.0 (CH), 31.5 (CH_2), 29.7 (CH_2), 28.2 (CH_2), 27.9 (3 x CH_3), 25.0 (CH_2), 24.8 (CH_2), 15.5 (CH_3), 10.8 (CH_3).

(3*R*)

^{1}H NMR (400MHz, $CDCl_3$) δ 7.38-7.16 (11H, m), 6.84 (1H, d, *J* 8.0), 6.21 (1H, s), 5.54 (1H, s), 5.51 (1H, d, *J* 8.3), 5.11-4.98 (1H, m), 5.09 (1H, d, *J* 12.5), 4.80 (1H, d, *J* 12.5), 4.75-4.61 (1H, m), 4.59 (1H, d, *J* 5.8), 4.50-4.29 (2H, m), 3.59-3.45 (1H, m), 3.29 (1H, dd, *J* 14., 3.9), 2.85 (1H, dd, *J* 14.0, 10.0), 2.29-2.12 (4H, m), 2.12-1.78 (6H, m), 1.46 (9H, s), 1.20-1.05 (1H, m), 0.93-0.83 (6H, m, 2 x CH_3).

IR (NaCl) υ_{max} 3290, 2925, 1728, 1648, 1537, 1452, 1367, 1247, 1157; FABHRMS (NBA/CsI) *m/e* 854.2775 ($[M+Cs]^+$, $C_{38}H_{51}N_5O_9$ requires 854.2741).

(3*S*) and (3*R*) 3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-[2'(*R*), 5'(*R*)-bis(methoxymethyl)-3'(*R*), 4'(*S*)-dimethoxypyrrolidine] 9

The coupling was carried out using the general peptide coupling method outlined above to give **26**, followed by oxidation using the general Dess-Martin oxidation procedure to give the desired product **9**. Flash chromatography eluting with 20% ethyl acetate in hexane gave the α-keto amide **9** (20 mg, quantitative). $R_f = 0.63$ (50% EtOAc in hexane).

All analysis performed on mixture of isomers:

NMR major isomer:

^{1}H NMR (400MHz, $CDCl_3$) δ 7.32-7.17 (10H, m), 5.75 (1H, d, *J* 8.7), 5.16-5.12 (1H, m), 5.09 (1H, d, *J* 12.4), 5.04 (1H, d, *J* 12.4), 4.39 (1H, dt, *J* 3.9, 8.3), 3.98-3.96 (1H, m), 3.92 (1H, t, *J* 6.0), 3.82-3.77 (1H, m), 3.73 (1H, dd, *J* 10.0, 5.0), 3.55 (1H, dd, *J* 10.0, 2.8), 3.47-3.17 (3H, m), 3.45 (3H, s), 3.40 (3H, s), 3.26 (3H, s), 3.19 (3H, s), 3.11 (1H, dd, *J* 14.1, 6.92); C^{13} NMR (100MHz, $CDCl_3$) δ 197.62 (C=O), 164.7 (C=O), 155.7 (C=O), 136.4 (C), 129.7 (2 x CH), 128.4 (2 x CH), 128.3 (4 x CH), 127.9 (CH), 126.7 (CH), 84.6 (CH), 83.6 (CH), 70.7 (CH_2), 69.7 (CH_2), 66.8 (CH_2), 60.7 (CH), 58.9 (CH), 58.7 (OCH_3), 58.7 (OCH_3), 58.4 (OCH_3), 58.4 (OCH_3), 56.7 (CH), 37.3 (CH_2).

minor isomer:

^{1}H NMR (400MHz, $CDCl_3$) δ 7.32-7.17 (10H, m), 5.46 (1H, d, *J* 9.0), 5.11-4.87 (3H, m), 4.56-3.17 (10H, m), 3.45 (3H, s), 3.38 (3H, s), 3.31 (3H, s), 3.21 (3H, s).

IR (NaCl) υ_{max} 2930, 1717, 1635, 1506, 1456, 1110; FABHRMS (NBA/CsI) *m/e* 661.1550 ($[M+Cs]^+$, $C_{28}H_{36}N_2O_8$ requires 661.1526).

2(*R*) (Methoxymethyl)-pyrrolidine 23

Spectral data:

^{1}H NMR (400MHz, CD_3OD) δ 3.80-3.70 (1H, m), 3.64 (1H, dd, *J* 10.6, 3.64), 3.50 (1H, dd, *J* 10.56, 7.76), 3.40 (3H, s), 2.16-1.98 (3 H, m), 32H, m), 1.81-1.74 (1H, m); ^{13}C NMR (100MHz, $CDCl_3$) δ 72.0 (CH_2), 60.7 (CH_3), 59.3 (CH), 46.6 (CH_2), 27.3 (CH_2), 24.8 (CH_2); FABHRMS (NBA/NaI) *m/e* 138.0902 ($[M+Na]^+$, $C_6H_{13}NO$ requires 138.0895).

2(*R*), 5(*R*)-Bis(hydroxymethyl)-3(*R*), 4(*S*)-dihydroxy-*N*-(Benzyloxycarbonyl)-pyrrolidine

A solution of 2(*R*), 5(*R*)-bis(hydroxymethyl)-3(*R*),4(*S*)-dihydroxypyrrolidine[44] (806 mg, 4.95 mmol) in H_2O (25 cm^3) was cooled to 0 °C in an ice bath and the pH was adjusted to 9-10 with Na_2CO_3 solution (0.3M). Benzyloxycarbonyl chloride (1.4 cm^3, 9.9 mmol, 2 eq.) was added dropwise and the solution was stirred 1 hour at 0 °C, and then 1 hour at ambient temperature. Solvent was removed *in vacuo*, and the residue was taken up in EtOAc, filtered, and concentrated *in vacuo*. Flash chromatography eluting initially with 50% ethyl acetate in hexane then 100% ethyl acetate gave 2(*R*), 5(*R*)-bis(hydroxymethyl)-3(*R*), 4(*S*)-dihydroxy-*N*-(Benzyloxycarbonyl)-pyrrolidine product as a pale yellow oil.(1.1g, 81%). R_f = 0.27 (EtOAc)

^{1}H NMR (400MHz, CD_3OD) δ 7.37-7.33 (5H, m), 5.13 (2H, s), 4.20-4.19 (1H, m), 4.13-4.10 (1H, m), 4.00 (1H, br s), 3.95-3.55 (5H, m); ^{13}C NMR (100MHz, CD_3OD) (two rotamers) δ137.8, 129.6,

129.2, 129.0, 77.8, 77.1, 68.4, 67.4, 66.7, 63.2, 62.2, 61.7, 61.6, 61.1; FABHRMS (NBA) *m/e* 320.1121 ($[M+Na]^+$ $C_{14}H_{19}NO_6$ requires 320.1110).

2(*R*), 5(*R*)-Bis(methoxymethyl)-3(*R*), 4(*S*)-dimethoxy-*N*-(Benzyloxycarbonyl)-pyrrolidine

To 2(*R*), 5(*R*)-bis(hydroxymethyl)-3(*R*), 4(*S*)-dihydroxy-*N*-(Benzyloxycarbonyl)-pyrrolidine (35 mg, 0.117 mmol) in dry THF (1 cm^3) was added CH_3I (116 μL, 1.86 mmol, 16 eq.) followed by NaH (60% dispersion in mineral oil) (28.1 mg, 6 eq.). The reaction mixture was stirred at ambient temperature for 20 h. and concentrated *in vacuo*. Flash chromatography eluting with 12% to 20% ethyl acetate in hexane gave 2(*R*), 5(*R*)-bis(methoxymethyl)-3(*R*), 4(*S*)-dimethoxy-*N*-(Benzyloxycarbonyl)-pyrrolidine as a colorless oil. (40mg, 97%). Rf = 0.6 (50% EtOAc in Hexane)

^1H NMR(400MHz, CD_3OD) δ 7.29-7.23 (5H, m), 5.04 (2H, br s), 4.10 (1H, br s), 3.74 (3H, br, s), 3.44-3.42 (4H, m), 3.34 (3H, br s), 3.31 (3H, br s), 3.23 (6H, br s); FABHRMS (NBA) *m/e* 376.1722 ($[M+Na]^+$ $C_{18}H_{27}NO_6$ requires 376.1736).

2(*R*), 5(*R*)-Bis(methoxymethyl)-3(*R*), 4(*S*)-(dimethoxy) pyrrolidine 23

To 2(*R*), 5(*R*)-bis(methoxymethyl)-3(*R*), 4(*S*)-dimethoxy-*N*-(Benzyloxycarbonyl)-pyrrolidine (40 mg, 0.181 mmol) in methanol (2 cm^3) was added Pd/C (10 mg). The mixture was stirred under a balloon of H_2 for 3 h. Filtration through celite followed by concentration *in vacuo* yielded the desired product **22** as a pale yellow oil (25 mg, quant.).

^{1}H NMR (250MHz, $CDCl_3$) δ 5.19 (1H, br s) 3.8-3.55 (8H, m), 3.54-3.35 (12H, m); ^{13}C NMR (62MHz, $CDCl_3$) 84.7, 83.7, 71.9, 69.1, 62.4, 59.9, 59.1, 59.0, 57.6, 57.5; FABHRMS (NBA) *m/e* 220.1543 ($[M+H]^+$ $C_{10}H_{21}NO_4$ requires 220.1549).

General procedure for coupling of epoxide to proline derivatives

To the pyrrolidine derivative **22**, **23**, **24**, **25** or **16** (20 mg, 0.091 mmol) was added dry methanol (2 cm^3), Cbz-phenylalanyl epoxide **21** (27 mg, 0.091 mmol, 1.0 eq.) and triethylamine (14 μL, 0.100mmol, 1.1eq.). The solution was refluxed for 32 h, and then concentrated *in vacuo*.

***N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2'(*R*) methoxymethyl]-pyrrolidine 6**

The pyrrolidine derivative **24** was coupled to the epoxide **20** as described in the general procedure to provide **6**. Flash chromatography eluting with ethyl acetate provided the desired product as a clear oil (40 mg, 56%). $R_f = 0.15$ (EtOAc).

^{1}H NMR (400MHz, CD_3OD) δ 7.29-7.16 (10H, m), 4.97 (1H, d, *J* 12.7), 4.92 (1H, d, *J* 12.7), 3.85-3.81 (1H, m), 3.77-3.73 (1H, m), 3.42-3.25 (3H, m), 3.31 (3H, s), 3.25-3.02 (2H, m), 2.89 (1H, m), 2.64 (1H, dd, *J* 13.8, 10.5), 2.61-2.52 (2H, m), 1.95-1.87 (1H, m), 1.84-1.77 (2H, m), 1.61-1.55 (1H, m); C^{13} NMR (100MHz, CD_3OD) δ 158.5 (C=O), 140.3 (2 x C), 130.5 (3 x CH), 129.4 (CH), 129.2 (3 x CH), 128.8 (CH), 128.5 (CH), 127.2 (CH), 73.0 (CH), 67.1 (2 x CH_2), 59.9 (CH_2), 59.3 (2 x CH_2), 57.5 (OCH_3), 57.2 (CH_2), 36.8 (CH_2), 28.5 (CH_2), 24.2 (CH_2); IR (NaCl) υ_{max} 3330, 2939, 1699, 1538, 1454, 1252, 1203, 1134, 699; FABHRMS (NBA/NaI) *m/e* 413.2458 ($[M+H]^+$, $C_{24}H_{32}N_2O_4$ requires 413.2440).

***N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2'(*R*), 5'(*R*)-bis(methoxymethyl)]-pyrrolidine 4**

The pyrrolidine derivative **22**[45] was coupled, according to the above conditions, to the epoxide **21** to provide **4**. Flash chromatography eluting with 30% to 50% ethyl acetate in hexane provided the desired product as a clear oil (35 mg, 40%). $R_f = 0.47$ (EtOAc/Hexane, 1:1).

^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.17 (10H, m), 5.02 (2H, dd, *J* 20.4, 12.1), 3.89 (1H, m), 3.50 (1H, m), 3.37 (3H, *s*), 3.31 (2H, d, *J* 1.1), 3.24 (3H, s), 3.20-3.18 (2H, m), 2.95-2.89 (4H, m), 2.85-2.75 (2H, m), 1.87-1.83 (2H, m), 1.54-1.52 (2H, m); ^{13}C NMR (100MHz, $CDCl_3$) δ 137.9, 129.6, 128.4, 128.2, 127.9, 127.8, 126.2, 77.5, 76.8, 71.2, 66.6, 66.3, 60.4, 59.0, 58.8, 54.9, 36.2, 29.6; FABHRMS (NBA) *m/e* 457.2689 ($[M+H]^+C_{26}H_{36}N_2O_5$ requires 457.2702)

***N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2'(*R*), 5'(*R*)-bis(methoxymethyl)-3'(*R*), 4'(*S*)-dimethoxy]-pyrrolidine 5**

The pyrrolidine derivative **23** was coupled to the epoxide **21** as described above. Flash chromatography eluting with 50% ethyl acetate in hexane gave the desired product **5** as a pale yellow oil (20mg, 42%) $R_f = 0.37$ (EtOAc/Hexane, 1:1).

^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.19 (10H, m), 5.02 (2H, dd, *J* 12.3, 20.6), 4.09 (1H, br s) 3.90 (1H, m), 3.70 (1H, d, *J* 3.7), 3.58 (1H, dd, *J* 6.4, 9.3), 3.54 (2H, m), 3.37 (3H, s), 3.36 (3H, s), 3.35 (3H, s), 3.40-3.28 (5H, m), 3.28 (3H, s), 3.25-3.17 (1H, m), 2.95-2.85 (2H, m), 2.82-2.77 (2H, m); ^{13}C NMR (100MHz, $CDCl_3$) δ 155.9, 137.9, 129.7, 128.4, 128.3, 127.9, 127.8, 126.3, 84.8, 83.9, 75.1, 72.2, 70.6, 69.9, 66.9, 66.4, 61.0, 59.0, 58.9, 58.0, 57.1, 54.9, 29.7; FABHRMS (NBA) *m/e* 517.2932 ($[M+H]^+$ $C_{28}H_{40}N_2O_7$ requires 517.2914).

_N_-[1-Phenyl-2(_S_)-[(benzyloxycarbonyl)amino]-3(_R_)-hydroxybutan-4-L-prolyl-_tert_-butyl amide 7

L-Proline *tert*-butyl amide **16** was coupled according to the above conditions to the epoxide **21** to give **7**. Flash chromatography eluting with 100% ethyl acetate in hexane gave the desired product **7** as a colorless oil (70mg, 48%). $R_f = 0.17$ (EtOAc/Hexane, 1:1).

^1H NMR (400MHz, $CDCl_3$) δ 7.33-7.14 (10H, m), 7.02 (1H, br s), 5.26 (1H, br s), 5.06 (2H, s), 3.93-3.86 (1H, m), 3.67-3.65 (1H, m), 3.29-3.16 (1H, m), 2.90-2.75 (3H, m), 2.67 (1H, d, *J* 1.7), 2.48 (1H, dd, *J* 4.8, 2.5), 2.20-2.05 (1H, m), 1.95-1.65 (3H, m), 1.30 (9H, s); ^{13}C NMR (63MHz, $CDCl_3$) δ 175.7, 174.4, 137.5, 129.2, 128.5, 128.4, 128.0, 127.9, 126.5, 72.1, 68.9, 66.7, 59.8, 56.1, 55.5, 50.4, 35.4, 30.9, 29.6, 28.6, 24.3; FABHRMS (NBA) *m/e* 468.2810 ($[M+H]^+$ $C_{27}H_{37}N_3O_4$ requires 467.2862).

_N_-[1-Phenyl-2(_S_)-[(benzyloxycarbonyl)amino]-3(_R_)-hydroxybutan-4-[2'(_R_)-(_tert_-butylamido)-4'(_S_)-methoxy]-pyrrolidine 8

The pyrrolidine derivative **25** (derived from *cis*-4-hydroxy-L-proline) was coupled to the epoxide **21** as described above. Flash chromatography eluting with 50% ethyl acetate in hexane gave the desired product **8** as a pale yellow oil (40mg, 60%) $R_f = 0.23$ (EtOAc).

^1H NMR (250MHz, $CDCl_3$) δ 7.34-7.14 (10H, m), 7.04 (1H, br s), 5.01 (2H, br s,), 4.81 (1H, d, *J* 8.8), 3.98-3.85 (1H, m), 3.84 (1H, t, *J* 3.7), 3.58 (1H, dd, *J* 6.0, 6.1), 3.35-3.25 (1H, m), 3.28 (3H, s), 3.20-3.12 (1H, m), 3.00-2.90 (2H, m), 2.85 (1H, dd, *J* 13.3, 8.1), 2.71 (1H, d, *J* 12.4), 2.66 (1H, d, *J* 12.4), 2.56 (1H, dd, *J* 10.3, 3.1), 2.30-2.10 (1H, m), 2.05-1.95 (1H, m), 1.33 (9H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 174.7 (C=O), 172.0 (C=O), 137.5 (C), 129.5 (2 x CH), 129.3 (C), 128.5 (3 x CH), 128.4 (2 x CH), 128.0 (CH), 127.9 (CH), 126.5 (CH), 79.9 (CH), 71.4 (CH), 68.3 (CH), 66.6 (C), 60.6 (CH_2), 59.7 (CH_2), 56.0 (CH_3O), 55.0 (CH), 50.3 (CH_2), 35.8 (CH_2), 29.7 (CH_2), 28.6 (3 x CH_3); FABHRMS (NBA/CsI) *m/e* 630.1970 ($[M+Cs]^+$ $C_{28}H_{39}N_3O_5$ requires 630.1944).

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